REMARKS

Favorable reconsideration of this application in light of the preceding amendments and the following remarks is respectfully requested.

Claims 6, 9 and 13 having been canceled, no claims having been added, and claims 1 and 2 having been rejoined for examination, Action at 2, the Applicant submits that claims 1-5, 7, 8 and 10-12 remain pending and properly under consideration in this application. The Applicant notes, however, that the Disposition of Claims as reflected in the Office Action Summary, does not accurately reflect the presently indicated rejoinder of claims 1 and 2.

The Applicant notes with appreciation the Examiner's acknowledgement that certified copies of all priority documents have been received by the USPTO. Action Summary at 12.

The Applicant notes with appreciation the Examiner's acknowledgement that the drawings filed with this application have been accepted by the Examiner. Action Summary at 10.

Specification

The Specification stands objected to for the incorporation of drawings depicting linearized plasmids, Action at 2. The Applicant submits that the amendments to the specification reflected above are sufficient to overcome these objections by replacing the drawings with corresponding text and incorporating appropriate references to the original FIGS. 4 and 5. The Applicant requests, therefore, that these objections be reconsidered and withdrawn accordingly.

Claim Objections

Claims 2 and 4 stand objected to for the incorporation of drawings depicting linearized plasmids, Action at 2. The Applicant submits that the amendments to the claims reflected above are sufficient to overcome these objections by replacing the drawings with corresponding textual descriptions of the linearized plasmids. The Applicant requests, therefore, that these objections be reconsidered and withdrawn accordingly.

Claims 1, 2, 3, 10 and 12 stand objected to for various informalities as detailed by the Examiner, Action at 3. The Applicants submit that the amendments to and/or cancellation of the claims reflected above are sufficient to overcome and/or render moot these objections. The Applicants request, therefore, that these objections be reconsidered and withdrawn accordingly.

Rejections under 35 U.S.C. § 112, first paragraph

Claim 1 stands rejected under 35 U.S.C. § 112, first paragraph, as not being enabled for plasmid constructions in which the promoter and the fluorescence-encoding gene are not operably linked.

The Applicant submits that the amendments to the claims reflected above, by which recitations of operable linkages have been added, are sufficient to overcome this rejection and request, therefore, that this rejection be reconsidered and withdrawn accordingly.

Rejections under 35 U.S.C. § 112, second paragraph

Claims 1-13 are rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to point out and distinctly claim the subject matter which Applicant regards as the invention. Action at 6-7.

With respect to claims 1 and 10, the Applicant submits that in this instance, the α-actin promoter was derived from zebrafish and incorporated in the plasmid construct p-αEGFPITR from which it was obtained for use in the disclosed gene fragments and methods and is not, therefore, limited to golden zebrafish. Substitute Specification ("Sub. Spec."), paragraph [0024]. The Applicant suggests, however, it is the functionality of the promoter rather than its specific source or structure that is being utilized in producing the transgenic golden zebrafish and would be recognized as such by those skilled in the art.

Accordingly, the Applicant suggests that "promoters designed for use in golden zebrafish," "promoters that work only in golden zebrafish," and "promoters isolated from golden zebrafish," Action at 6, would each be recognized by those skilled in the art as capable of providing the necessary functionality. Accordingly, the Applicant suggests that while broad, the term "promoter of golden zebrafish," is not unclear. The Applicant submits that alternative language, specifically "an α-actin gene promoter capable of activity in golden zebrafish," has been presented in claim 12. To the extent that the Examiner finds this alternative language more acceptable, the Applicant authorizes an Examiner's amendment to introduce this language into claims 1 and 10.

With respect to claim 3 and the term "eggs," the Applicant submits that the amendments to the claims reflected above make clear that the incubated eggs of interest are those that have developed into "embryos." Sub. Spec. at paragraph [0024]

The Applicant submits, therefore, that the amendments to the claims reflected above are sufficient to overcome these rejection and request that they be reconsidered and withdrawn accordingly.

Rejections under 35 U.S.C. § 102

Claims 1 and 2 stand rejected under 35 U.S.C. § 102(b) as anticipated by Chou et al.'s (*Transgenic Research*, 10:303-315, August 2001, IDS) ("Chou") or Hsiao et al. (*Developmental Dynamics*, 22:323-336, April 2001) ("Hsiao"). The Applicant respectfully traverses this rejection for the reasons detailed below.

The Applicant submits that the amendments reflected above to claims 1 and 2 are sufficient to distinguish the claimed gene fragment from those disclosed in Chou and Hsiao, specifically by removing the pα-EGFPITR (8.1 kb) segment from the claimed gene fragment. The Applicant requests, therefore, that this rejection be reconsidered and withdrawn accordingly.

Rejections under 35 U.S.C. § 103

Claims 1-4, 6, 7, 10 and 11 stand rejected under 35 U.S.C. § 103(a) as unpatentable over Hsiao in view of Carvan et al. (Ann. N.Y. Acad. Sci. 919:133-147). The Applicants respectfully traverse this rejection for the reasons detailed below.

Although Hsiao does teach the use of EGFP, the Applicant submits that Hsiao did not teach or suggest wider applicability and clearly did not suggest that all fluorescent genes were incorporated. The Applicant notes that Rahman et al., for example, disclosed that the ubiquitous expression characteristics of the β-actin gene promoter were not observed when a range of β-actin/*lacZ* fusion gene constructs were used in lines of transgenic mice as noted on page 417, column 2, and lines 8-12 (*Transgenic Research* 9:417–427); page 418, column 1, and lines 15-21 (*Transgenic Research* **9:** 417–427) ("Rahman") (copy attached). Accordingly, the Applicant submits that it is clear from the art that whether or not a particular gene will be expressed and at what level it will be expressed remains unpredictable, even when they are driven by the same promoter.

The Applicant submits that the present claims are directed to the successful generation of viable red fluorescent adult zebrafish that were not taught or suggested in the prior art. The Applicant maintains, therefore, that achieving expression of a red fluorescent gene product in adult zebrafish is both novel and not obvious.

As detailed in the attached Declaration, the Applicant maintains that the replacement of EGFP with RFP in zebrafish is not easily derived by one skilled in the art from Hsiao's disclosure. Even assuming one skilled in the art was motivated to combine Hsaio and Carvan in the manner suggested to make the fluorescent transgenic fish, given the uncertainly of gene expression, one skilled in the art could not have reasonably entertained confidence in the likelihood of success of such a combination in obtaining an adult transgenic fish expressing systemic red fluorescence.

The Applicant also disputes the contention that visible systemic fluorescence in adult fish is an inherent feature of transgenic fish that incorporate a gene encoding a fluorescent gene product. Indeed, although golden zebrafish recited in the claims and the leopard zebrafish used by Hsiao, are considered to be the same species, it does not automatically follow that the two strains will express fluorescence in an identical or even similar manner. Indeed, even subtly different genetic configurations can produce widely differing effects on transgene expression, a fact that has been attributed to the presence of strain-specific modifiers of expression as noted by Opsahl et al. at page 1107, column 2, line 7-9 and column 2, line 18-22 (*Genetics* **160**: 1107-1112) ("Opsahl") (copy attached).

Furthermore, the Applicant maintains that analysis of expression of a gene on a tissue-to-tissue basis demonstrated that gene expression in stably transformed fish occurred with variable intensity in different organs and tissues and was also sometimes variable in different cells as noted in Rahman's Table 1 and Figures 2 and 5. Further, the Applicant notes that those skilled in the art will appreciate that the expression of transgene may be influenced by the promoter driving the transgene, the copy number of the transgenes in the genome, and by interaction between transgene and flanking sequence DNA as noted in Rahman, at page 417, column 1, and lines 1-7. Thus, the Applicant maintains that the lack of predictability in the expression of transgenes is sufficient to rebut any suggestion of any inherent result of a proposed combination. The Applicant, therefore, submits that the production of red fluorescent zebrafish in which the fluorescence is expressed throughout the whole body could not be predicted by the combination of the applied references and remains patentable over these references.

With respect to the Examiner's suggestion that none of the citations suggests that fluorescence cannot be observed in adult fish and there is no evidence that the presence of scales would block fluorescence, Action at 10, the Applicant contends that the Examiner's suggestion is based on mere speculation, not any identified teaching in an applied reference. The Applicant contends that prior work has shown that, contrary to the Examiner's suggestion, that fluorescence may be expressed in transgenic fish at the embryo and/or juvenile stages and still be lacking in adult fish. The Applicant contends that persons skilled in the art will appreciate that because fish's scales cover its skeletal muscle, the color of the scales directly blocking the fluorescence expressed in the skeletal muscle of the fish will definitely effect the ornamental value. To the contrary, the use of Hsaio teaching can produce the systemic fluorescent fish.

Except of different fluorescent gene from Hsaio, the present invention uses similar Hsaio teaching to produce systemic red fluorescent zebrafish. In general, persons skilled in art may consider that the replacement of EGFP with RFP is easily and does not have any nonobviousness. However, as noted above, expression of a transgene is known to be influenced by the interaction between transgene and flanking sequence DNA. Further, as stated in the Applicant's Declaration (provided herewith), an extended effort involving much experimentation was required to produce the fluorescent fish of the present invention. The Applicant maintains that the numerous unsuccessful attempts endured by one of more that ordinary skill in the art with knowledge of the prior art is additional evidence of the non-obviousness of the particular combination with which success was achieved.

The Applicant also notes that the claims have been amended to limit the claimed fluorescent gene to a red fluorescent gene in present invention and, with respect to those claims to the transgenic fish, have been limited to adult fish which exhibit systemic expression of the red fluorescence gene.

The Applicant also contends that while Finley did teach the use of DsRed in zebrafish, the Applicant notes that red fluorescence expression in zebrafish disclosed by Finley is driven by Xenopus EF1α promoter and that Finley only recorded fluorescent phenotype at early developmental stage up to 24-dpf embryo and did not provide any data with respect to adult fish. In addition, the Applicants contend that red fluorescence expression of DsRed driven by Xenopus EF1α promoter is more in discrete or patchy groups in embryo (as detailed in Finley et al., Biotechniques 31:66-72) rather than in a systemic expression pattern as in present invention.

The Applicant has prepared the following TABLE to illustrate the differences between the pending claims and the teachings of the applied references.

TABLE

Pending Claims	Hsiao	Carven	Finley
α-actin promoter	β-actin promoter	CMV promoter EF1α promoter	Xenopus EF1α promoter
Larval and adult fish with **red** fluorescence	Larval and adult fish with **green** fluorescence	Larval and adult fish with **green** fluorescence	Larval fish with **red** fluorescence
Systemic fluorescence	Systemic fluorescence	Mosaic fluorescence	Mosaic fluorescence
Ornamental use	Academic use	Academic use	Academic use

As reflected in the TABLE, no combination of the applied referenced Hsiao, Carven and Finley teaches a combination including use of the α-actin promoter, to produce an adult fish exhibiting systemic red fluorescence. Although one reading Hsiao, Carven and Finley in the manner suggested may conclude that the systemic or mosaic green fluorescent fish can be prepared by the using a combination of a β-actin promoter and RFP in golden zebrafish. However, in light of the demonstrated uncertainty in the outcome of transgenic organisms, particularly with regard to the expression of the transgene(s), the Applicant maintains that the information identified in the applied references would not be sufficient to lead one skilled in the art to believe, with any reasonable degree of certainty, that a systemic red fluorescent could be obtained by using an α-actin promoter (different promoter from the combination) and RFP.

CONCLUSION

In view of the above remarks and amendments, the Applicants respectfully submit that each of the pending objections and rejections have been addressed and overcome, leaving the present application in condition for allowance. A notice to that effect is respectfully requested.

If the Examiner believes that personal communication will expedite prosecution of this application, the Examiner is invited to contact the undersigned.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies to charge any underpayment or non-payment of any fees required under 37

C.F.R. §§ 1.16 or 1.17, or credit any overpayment of such fees, to Deposit Account No. 08-0750, including, in particular, extension of time fees.

Respectfully submitted,

HARNESS, DICKEY & PIERCE, P.L.C.

By: _____
John A. Castellano
Reg. No. 35,094

P.O. Box 8910
Reston, VA 20195
(703) 668-8000

JAC/GPB

Attachments: Declaration of Huai-Jen TSAI
Opsahl Article
Rahman Article